PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
344421/D20110 BC		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/IB 03/00986	25.02.2003	25.02.2002
International Patent Classification (IPC) or both national classification and IPC		
C12Q1/68, C12Q1/68		
Applicant		
INSTITUT PASTEUR et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 10 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
12.09.2003	07.05.2004
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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/IB 03/00986

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-41 as originally filed

Sequence listings part of the description, Pages

1-34 as originally filed

Claims, Numbers

1-59 as originally filed

Drawings, Sheets

1/6-6/6 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☒ contained in the international application in written form.

   ☒ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority in written form.

   ☐ furnished subsequently to this Authority in computer readable form.

   ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☐ the claims, Nos.:

   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application,

   ☒ claims Nos. 6,27-29,51,52,58,59 (all partially); 32,33,36,39,43,54 (all completely)

   because:

   ☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (specify):

   ☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

   ☒ the claims, or said claims Nos. 32,33,36,39,43,54 (all completely) are so inadequately supported by the description that no meaningful opinion could be formed.

   ☒ no international search report has been established for the said claims Nos. 6,27-29,51,52,58,59 (all partially)

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the Standard.

   ☐ the computer readable form has not been furnished or does not comply with the Standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	2-14,18-31,34,35,37,38,40-42,44-52,55,59
	No:	Claims	1,15-17,53
Inventive step (IS)	Yes:	Claims	6-8,10-14,20,22-28,31,34,35,37,38,40,41,44-50,55-59
	No:	Claims	1-5,9,15-19,21,29,30,42,51-53
Industrial applicability (IA)	Yes:	Claims	1-31,34,35,37,38,40-42,44-53,55.59
	No:	Claims	

2. Citations and explanations

see separate sheet

Re item III:

1.1 The expression of proteins encoded by nucleic acids comprising the TbD1 deletion in vivo is purely speculative and not supported by any experimental evidence whatsoever. Hence, neither a method based on the detection of antibodies produced in vivo against said proteins (claim 32) nor a method wherein the presence of said proteins in vivo is determined (claim 36) is not supported by the description (Art. 6 PCT) which does not disclose these feature of the invention in a way sufficiently clear and complete for it to be carried out by the skilled person (Art. 5 PCT).

1.2 The same applies to a method of detecting antibodies directed against a subgroup of M. tuberculosis (claim 43) (Art. 5 and 6 PCT).

1.3 No support is provided for a vaccination/immunisation with Mycobacteria of the Mycobacteria tuberculosis complex (MTC) complex devoid of the TbD1 deletion eliciting a T cell response against the hypothetical proteins encoded by the sequence of the said deletion. Hence, the method according to claim 33 which in which such a T cell response is monitored lacks support by the description (Art. 6 PCT) and is furthermore not disclosed in a way sufficiently clear and complete for it to be carried out by the skilled person (Art. 5 PCT).

1.4 No support is provided for the proteins referred to in claims 39 and 54 eliciting a protective immunity on which said claims to vaccines are based (Art. 6 PCT).

1.5 In consequence of the foregoing considerations, no opinion will be given on novelty, inventive step and industrial applicability of claims 32, 33, 36, 39, 43 and 54.

Re item V:

1. Reference is made to the following documents.
   D1: COLE S T ET AL: "Deciphering the biology of Mycobacterium tuberculosis from the complete genome sequence" NATURE, MACMILLAN JOURNALS LTD. LONDON, GB, vol. 393, 11 June 1998 (1998-06-11), pages 537-544 & Genbank accession no. NC_000962
   D2: DATABASE GENBANK [Online] NCBI; 3 August 2001 (2001-08-03) COLE S.T. ET AL.: "Mycobacterium tuberculosis H37Rv complete genome;

segment 69/162" retrieved from HTTP://WWW.NCBI.NLM.NIH.GOV Database accession no. Z74020

D3: WO 00 55362 A (BILLAULT ALAIN ;COLE STEWART (FR); GARNIER THIERRY (FR); GORDON ST) 21 September 2000 (2000-09-21)

D4: US 6 291 190 B1 (BEHR MARCEL ET AL) 18 September 2001 (2001-09-18)

D5: BROSCH R ET AL: 'A new evolutionary scenario for the Mycobacterium tuberculosis complex.' PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES, vol. 99, no. 6, 19 March 2002 (2002-03-19), pages 3684-3689

2.1 Claim 1 relates to a nucleic acid represented by SEQ ID No. 1, part of which is deleted in a subgroup of M. tuberculosis. As the said nucleic acid, however also includes other sequences which are not deleted in said Mycobacteria and said claim further proposes nucleic acids which hybridise to the said nucleic acid i.e. which also hybridise to sequences of SEQ ID No. 1 which are not deleted in M. tuberculosis, the nucleic acids disclosed in D1, namely Genbank accession no. NC_000962 (cf. the whole document) and D2 (cf. the whole document) fall within the scope of claim 1, thus destroying its novelty (Art. 33(2) PCT).

2.2 D1 and D2 further disclose polypeptides encoded by the said nucleic acids (supra), thereby taking away the novelty of claim 15 (Art. 33(2) PCT).

2.3 D1 as well as D2 teach a polypeptide consisting of SEQ ID No. 22, as well as an isolated nucleic acid encoding the said (supra). Hence, the subject-matter of claim 16 and 17 lacks novelty over either D1 or D2 (Art. 33(2) PCT).

2.4 The only constituent of the immunogenic composition according to claim 53 is a polypeptide according to SEQ ID No. 22. Hence, said claim also lacks novelty over either D1 or D2 (supra)(Art. 33(2) PCT).

2.5 The subject-matter of claims 6-8, 10-14, 20, 22-28, 31, 34, 35, 37, 38, 40, 41, 44-50, 55-59 is considered novel as the combination of features suggested by said claims is not proposed by any of the prior art documents at hand (Art. 33(2) PCT).

3.1 As the nucleic acid proposed by claim 2 includes nucleic acids which are present in all M. tuberculosis and nucleic acids of M. tuberculosis are already known from D1 and D2 (supra), the problem to be solved by said claim has to be regarded as

the provision of further nucleic acid probes for M. tuberculosis. In view of D1 and D2 which disclose nucleic acids of M. tuberculosis, the nucleic acid proposed by said claim is considered to represent a mere choice of subsequences out of equally likely alternatives which involves nothing but routine experimentation and does not require an inventive step (Art. 33(3) PCT).

3.2 The same considerations also apply to claims 3-5 and 9 which are therefore considered not inventive (Art. 33(3) PCT).

3.3 D1 and D2 teach inter alia the subsequence of the M. tuberculosis genome encoding the polypeptide of SEQ ID No. 22 (supra). Isolating the said subsequence out of the nucleic acids disclosed in D1 and D2 requires routine experimentation and produces no unforeseeable effect. Hence, the subject-matter of claim 18 is considered not inventive (Art. 33(3) PCT).

3.4 The insertion of a known nucleic acid into a recombinant vector (claim 19), the establishment of a recombinant cell comprising a known nucleic acid or the said vector (claim 21), as well as the use of a known nucleic acid for the amplification/detection of inter alia the genome from which it is derived (claims 29 and 30) involve routine experimentation without producing any unforeseeable effect and therefore do not establish an inventive step. Hence, claims 19, 21, 29 and 30 are not considered inventive (Art. 33(3) PCT).

3.5 Not every kit falling under the scope of claims 42, 51 and 52 solves the problem posed, namely of allowing the detection and in particular the distinction of certain M. tuberculosis from other Mycobacteria of the MTC. Only the combination of certain markers allows the identification of M. tuberculosis and M. africanum. The other Mycobacteria of the MTC cannot be identified. Hence, the kits proposed by claims 42 and 51 do not appear to solve the said problem, but merely provide further reagents for the detection of Mycobacteria of the MTC.
The primers according to claims 42(a), 51(a) and 52(a) are already known from D1 and D2 (cf V 2.1 supra). The addition of other reagents namely for the amplification of nucleic acid sequences requires routine skills and does not establish an inventive step. Hence, the subject-matter of claim 42 is not inventive (Art. 33(3) PCT).
As regards, claims 51 and 52, the additional primers suggested for the detection of RD4 and RD9 are a mere choice out of equally likely alternatives in view of the disclosure of said markers in D3 and D4. The said additional primers are merely

juxtaposed to the other primers without producing any non-obvious inter-relation. Hence, the subject-matter of claims 51 and 52 is considered not inventive (Art. 33(3) PCT).

4.1 The subject-matter of claims 6-8 and 10-14 insofar as it is clearly defined i.e. not referring to hybridising nucleic acids (cf. V 5.1 infra) relates to nucleic acids which are distinct from the prior art represented by any of D1 or D2 in that they comprise sequences of the TbD1 deletion or comprise subsequences thereof. Hence, they allow the detection of the said deletion and thereby the identification of a subgroup of M. tuberculosis. The technical problem thus consists of providing nucleic acids that allow the identification of a subgroup of M. tuberculosis. As none of the prior art documents provides a solution therefore, the subject-matter of said claims is considered to involve an inventive step (Art. 33(3) PCT).

4.2 The same applies to claims 20 and 22 which provide a defined clone of a nucleic acid comprising the said deletion (Art. 33(3) PCT).

4.3 Similar considerations also apply to methods based on the detection of said deletion (claims 23-27, 40, 41 and 47-50), to proteins encoded by the sequence of said deletion and products comprising the said proteins (claims 31 and 38), to antibodies specific for said proteins (claim 32), to kits comprising said antibodies (claim 37), to kits for the detection of said deletion (claims 28 and 34), to the use of said deletion (claims 44 and 46), as well as to reagents which comprise sequences specific for the deletion or proteins encoded by the said (claims 58 and 59). Hence, claims 23-28, 31, 34, 35, 37, 38, 40, 41, 44, 46-50, 58 and 59 are regarded inventive (Art. 33(3) PCT).

4.3 Claims 45 and 55 propose the use of the mmpL6^{551} polymorphism as a genetic marker for the differentiation of Mycobacteria of the MTB. The mmpL6^{551}AAG polymorphism is a marker for M. bovis and M. microti. None of the prior art documents at hand, neither taken alone, nor taken in combination suggests this polymorphism to be used for the said differentiation (Art. 33(3) PCT).

4.4 Similar considerations also apply to claims 56 and 57 which propose a probe for the detection of M. canettii, as well as its use as a genetic marker (Art. 33(3) PCT). The suggested probe identifies an insertion unique to the genome of M. canettii.

5. Industrial applicability of claims 1-31, 34, 35, 37, 38, 40-42, 44-53, 55 and 59 is acknowledged (Art. 33(4) PCT).

6.1 The wording "nucleic acid that hybridises ... to SEQ ID No. 1/4" used in claims 1 and 7 respectively gives way to an undeterminable number of possible nucleic acids, thereby rendering the scope of said claim unclear (Art. 6 PCT). The same consideration applies to the subject-matter of dependent claims 2-6 and 8-14 insofar as it refers to said hybridising nucleic acids.

6.2 Claims 6, 9, 28, 34, 37, 42, 51 and 52 do not meet the requirements of Art. 6 PCT in that the matter for which protection is sought is not clearly defined. The claims attempt to define the subject-matter in terms of the result to be achieved ("specifically deleted../present in", "susceptible to be used...", "necessary to...", "allowing...", "suitable for...") which merely amounts to a statement of the underlying problem. The technical features necessary for achieving this result should have been added.

6.3 Furthermore, the definition of the nucleic acid fragment referred to in claim 6 is not clear, as at least certain M. africanum isolates also contain the TbD1 deletion (cf p. 29, l. 20-28 of the present application). Consequently, the subject-matter of claim 6 is not clearly defined (Art. 6 PCT).

6.4 Claims 23 and 40 appear to relate to the same subject-matter. Hence, either of said claims is considered superfluous (conciseness: Art. 6 PCT).

6.5 The terms "TbD1 deletion", "TbD1 genetic marker" and "specific insertion element of M. canettii" used in claims 44, 46, 47, 51, 55, 58 and 59 are vague and unclear and leave the reader in doubt as to the technical features to which they refer thereby rendering the scope of said claims unclear (Art. 6 PCT). The terms "TbD1 deletion" and "TbD1 genetic marker" were interpreted as referring to SEQ ID No. 4, whereas the term "specific insertion element of M. canettii" was considered to refer to nucleotides 399-2378 of SEQ ID No. 19.

6.6 The primers referred to in claims 27-29 are defined by reference to claim 25 which, however, does not provide a definition of the said primers, and are therefore unclear. Consequently, the scope of claims 27-29 is not clear (Art. 6 PCT).

6.7 It is clear from the description as a whole that the detection of the presence or absence of the TbD1 deletion is an essential step of the methods according to claims 27 and 41.
Since independent claims 27 and 41 do not contain this feature they do not meet the requirement following from Art. 6 PCT taken in combination with R. 6.3(b) PCT that any independent claim must contain all the technical features essential to the definition of the invention.

6.8 The only technical feature suggested by claim 53 is identical to that of claim 16. Hence, both claims are indistinguishable and claim 53 therefore appears to be superfluous (conciseness: Art. 6 PCT).

6.9 For the same reasons claim 38 appears to be superfluous in view of claim 31 (conciseness: Art. 6 PCT).

6.10 Claims 23-27 propose methods for the discriminatory detection of inter alia a subgroup of M. tuberculosis versus M. africanum based on the detection of the TbD1 deletion. D5, however, teaches that at least certain isolates of M. africanum also contain the said deletion (p. 3687, c. 1, § 3 - c. 2, § 1). Hence, as regards the distinction of the said latter M. africanum isolates from the said subgroup of M. tuberculosis the methods suggested by the said claims appear to lack reproducibility (Art. 5 PCT).

6.11 Claim 57 relates to the use of a nucleic acid referred to in claim 53. Claim 53, however, does not relate to a nucleic acid, but an immunogenic composition. Hence, the definition of the subject-matter of claim 57 is unclear (Art. 6 PCT). For the purpose of examination said claim has been interpreted as referring to claim 56.